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Research article

Inhibition of telomerase causes vulnerability to endoplasmic reticulum stress-induced neuronal cell death

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HIGHLIGHTS

- Telomerase inhibitor, BIBR1532, attenuated ER stress resistance in MCF7-TERT cells.
- TERT was expressed in SH-SY5Y human neuroblastoma cell lines.
- BIBR1532 enhanced ER stress-induced neuronal cell death of SH-SY5Y cells.
- Telomerase may enhance vulnerability of ER stress-induced neurodegeneration.

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ABSTRACT

Endoplasmic reticulum (ER) stress is implicated in several diseases, such as cancer and neurodegenerative diseases. In the present study, we investigated the possible involvement of telomerase in ER stress-induced cell death. ER stress-induced cell death was ameliorated in telomerase reverse transcriptase (TERT) over-expressing MCF7 cells (MCF7-TERT cell). Telomerase specific inhibitor, BIBR1532, reversed the inhibitory effect of TERT on ER stress-induced cell death in MCF7-TERT cells. These findings suggest that BIBR1532 may specifically inhibit telomerase activity, thereby inducing cell death in ER stress-exposed cells. TERT was expressed in the SH-SY5Y neuroblastoma cell line. To analyze the possible involvement of telomerase in ER stress-induced neuronal cell death, we treated SH-SY5Y neuroblastoma cells with BIBR1532 and analyzed ER stress-induced cell death. We found that BIBR1532 significantly enhanced the ER stress-induced neuronal cell death. These findings suggest that inhibition of telomerase activity may enhance vulnerability to neuronal cell death caused by ER stress.

1. Introduction

The endoplasmic reticulum (ER) is an organelle involved in assisting protein folding. Recent observations suggest that disruption of ER function leads to accumulation of unfolded proteins in the ER and causes ER stress-induced cell death [21]. Accumulating evidence indicates the possible involvement of ER stress in several types of diseases, including cancer, neurodegenerative diseases, diabetes and obesity [9,12]. However, the underlying mechanisms of ER stress-induced cell death are yet to be fully understood.

Telomerase is an enzyme involved in adding 5′-TTAGGG-3′ at the 3′-end of eukaryotic chromosomal DNA [6]. Telomerase is made up of several complex components. Telomerase reverse transcriptase (TERT) is one of the components of telomerase and plays an important role in its activation [2]. Interestingly, recent observation indicates that TERT stimulates cell survival independently, through its enzyme activity [1]. Also, TERT was expressed in neurons and protects against oxidative damage [18]. Furthermore, TERT was shown to protect against amyloid β-induced neuronal cell death [22], suggesting a possible role of TERT in preventing neuronal cell death in Alzheimer's disease (AD).

The involvement of ER stress in the progression of neurodegenerative diseases, such as AD, has been reported in several studies [13,14]. ER resident caspase-12-deficient mice were shown to be

Abbreviations: ER stress, endoplasmic reticulum stress; TERT, telomerase reverse transcriptase.

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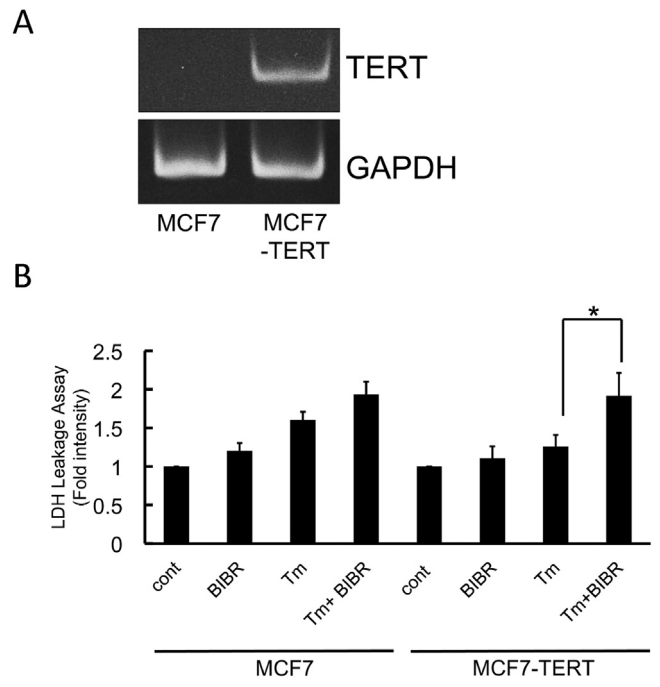

Fig. 1. Involvement of telomerase in ER stress-induced cell death.
(A) Over expression of TERT in MCF7-TERT cell lines. TERT mRNA was analyzed by RT-PCR using specific primers for human TERT. TERT was over expressed in MCF7-TERT cell lines compared with normal MCF7 cell lines. (B) Tunicamycin (Tm, 10 μg/mL, 72 h) caused cell death in MCF7, but the tunicamycin-induced cell death was attenuated in MCF7-TERT cell lines. On the other hand, treatment with telomerase inhibitor (BIBR1532, 50 μM) caused cell death in tunicamycin-exposed MCF7-TERT cell lines. Cell death was analyzed using an LDH leakage assay. $^{**}p < 0.01$, n = 7.

resistant against neuronal cell death caused by amyloid β [16]. Presenilin-1 mutation-mediated pathology of familial Alzheimer's disease (FAD) was shown to be linked with ER stress [15]. However, the possible link between ER stress and TERT in neurodegeneration is still unknown. Thus, in the present study, we examined the possible role of TERT in ER stress-induced neuronal cell death.

2. Materials and methods

2.1. Materials and reagents

Tunicamycin was obtained from Wako Pure Chemical Industries, Ltd. (Japan). BIBR1532 was obtained from Adooq BioScience (CA).

2.2. Cell culture

The MCF-7 human breast cancer cell line was cultured in DMEM with 10% fetal calf serum (FCS), 100 units/ml of penicillin G, 100 μg/mL of streptomycin, and 0.25 μg/mL of amphotericin B (nacalai tesque, Japan). The SH-SY5Y human neuroblastoma cell line was cultured in DMEM with 10% FCS. Cells were kept at 37 °C in 5% CO_2/95% air.

2.3. Transduction of TERT in the MCF7 cell line

Transduction of *TERT* in the MCF7 cell line was performed as previously described [11]. TERT over expression in a hTERT-transduced clone was checked by RT-PCR (28 cycles) using specific primers for human TERT (Fig. 1A).

2.4. Reverse transcription-polymerase chain reaction (RT-PCR)

Total RNA was isolated using TriPure Isolation Reagent (Roche Molecular Biochemicals, Indianapolis, IN). RT-PCR was performed as previously described, with a few modifications [10]. Specifically, cDNA was synthesized from the total RNA by reverse transcription using ReverTra Ace (Toyobo, Japan), Oligo $(dt)_{16}$ primer (eurofins) in a 20 μl reaction mixture containing RT buffer (Toyobo, Japan), 1 mM dNTP mix, 10 mM dithiothreitol (DTT), and 40 U of RNase inhibitor (enzymatics, MA). Total RNA and Oligo (dt_{16} primers were incubated at 70 °C for 10 min prior to reverse transcription. After incubation for 1.5 h at 46 °C, the RT reaction was terminated by denaturing the reverse transcriptase for 5 min at 100 °C. For PCR amplification of GAPDH, 1 μl of cDNA was added to 10.5 μl of a reaction mix containing each primer, dNTP mix, Taq polymerase (Expand High Fidelity; Roche), and reaction buffer. For PCR amplification of TERT, 1 μl of cDNA was added to 10.5 μl of a reaction mix containing each primer, dNTP mix, Taq polymerase (LA Taq polymerase; Takara, Japan), and reaction buffer (GC buffer). PCR was performed in a DNA Thermal Cycler (Applied Biosystems veriti or Takara Dice). The following primers were used: TERT upstream, 5′-tct ttg ggg tct tgc ggc tga a-3′; TERT downstream, 5′-gcg tct ggg ctg tcc tga gtg a-3′; GAPDH upstream, 5′-aaa ccc atc acc atc ttc cag-3′; and GAPDH downstream, 5′-agg ggc cat cca cag tct tct-3′. The PCR products (10 μl) were resolved by electrophoresis in an 8% polyacrylamide gel in TBE buffer. The gels were stained with ethidium bromide, and then photographed under ultraviolet light.

2.5. Lactate dehydrogenase leakage assay

The viability of cells was estimated by the lactate dehydrogenase (LDH) leakage method using a cytotoxicity detection kit (Roche Molecular Biochemicals, Indianapolis, IN), according to the manufacturer's protocol. LDH activity was measured as the optimal density at 492 nm.

2.6. Statistics

Results are expressed as the mean ± S.E.. Statistical analyses were performed using Paired *t*-tests.

3. Results and discussion

3.1. Telomerase inhibitor reversed TERT mediated resistance against ER stress

To analyze the involvement of telomerase on ER stress-induced cell death, we treated normal and TERT over-expressed MCF7 cell lines (MCF7-TERT cells) with a ER stress inducer (tunicamycin). Using RT-PCR, we confirmed that TERT was over markedly expressed in MCF7-TERT cells compared with normal MCF7 cells (Fig. 1A). In agreement with a previous report [11], we observed that ER stress-induced cell death was ameliorated in the MCF7-TERT cell line (Fig. 1B). We next tested the possibility that telomerase specific inhibitor, BIBR1532, may be able to reverse the inhibitory effect of ER stress-induced cell death in MCF7-TERT cells. BIBR1532 is a highly selective inhibitor of telomerase [3,17]. In MCF7 cells, BIBR1532 alone caused 1.2-fold cell death. ER stress inducer (tunicamycin) caused 1.6-fold cell death. Tunicamycin + BIBR1532 caused 1.9-fold cell death. Therefore, the effect of tunicamycin + BIBR1532 on MCF7 cell death was additive. On the other hand, these effects are different at MCF7-TERT cell line, which showed synergistic cell death (BIBR1532: 1.1-fold, tunicamycin: 1.3-fold, tunicamycin + BIBR1532: 1.9-fold of cell death at MCF7-TERT cell line) (Fig. 1B). These findings suggest that telomerase